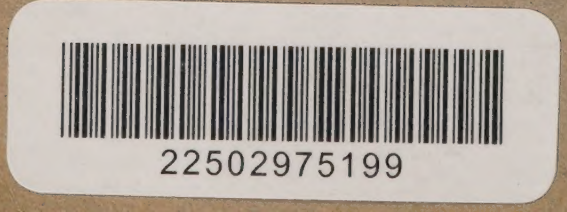
THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

# THE DIRECTOR'S TWENTY-SIXTH ANNUAL REPORT 1944-45



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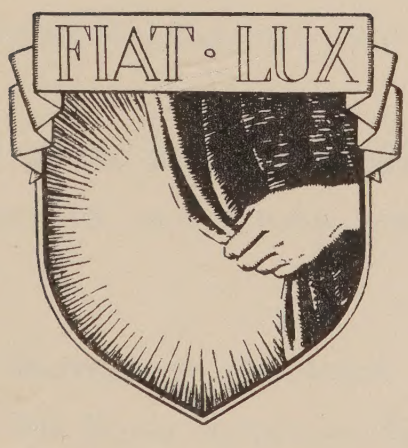


Melbourne:
Spectator Publishing Co. Pty. Ltd., Printers, Melbourne.
1945

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*Attached or Visiting Workers During* 1944-1945.

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Major J. F. Funder, B.Sc. (Adel.), M.B., B.S. (Adel.), A.A.M.C.
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Mrs. J. McEwin, B.Sc., Institute of Medical and Veterinary Science, Adelaide.
W. C. Boake, B.Sc. (Melb.).

# History

The Walter and Eliza Hall Institute was founded in 1916 largely on the initiative of the late Sir Harry Allen, then Professor of Pathology in the University of Melbourne. He was associated with members of the medical staff of the Melbourne Hospital in urging the necessity for ensuring that a certain amount of clinical research should be associated with the development of modern diagnostic laboratories in the hospital, then on the Lonsdale Street site. As a result of their representations the trustees of the late Walter and Eliza Hall agreed to complete the pathological block of the old hospital to provide accommodation for an institute of research in pathology and medicine, and to make annual payments of £2500 towards its upkeep; this was subsequently increased to £3200 annually. It was this contribution of the trustees which allowed the foundation of the Institute, and which has provided the sheet anchor of its support through the years, but ever since its inception the Institute has attracted a widening range of financial support from other official and private sources.

The first director-designate of the Institute was the late Dr. G. C. Mathison, whose death from wounds received on Gallipoli, at the age of 31, ended a career of brilliant promise. The actual work of the Institute was commenced in 1920 under the direction of Dr. S. W. Patterson. He resigned in 1923, and was succeeded by Dr. C. H. Kellaway in August of the same year.

The development of the Institute to its present status will always be linked with Dr. Kellaway's name. For twenty years he was responsible both for the expanding range of scientific work in the laboratories, and for obtaining the necessary financial support for such expansion. During the period of Dr. Kellaway's directorship, and up to the present, the Institute has received substantial help from the University, and from some of the important Victorian charitable trusts, notably the Truby Williams Trust, which has provided £1000 in each of the past two years. From 1934 to 1938 the Rockefeller Foundation gave £1000 per annum to help in the development of the virus department. To continue this work Mr. E. Alex Cato generously contributed a similar amount for five years, 1939 to 1943.

In addition to this help from private sources an increasing amount of support has been obtained from the Commonwealth Government, at first through the Department of Health, and since 1937 from the National Health and Medical Research Council. This help has been essential to the expansion of the Institute's work.

Other important contributions to the Institute have been the bequests of Mrs. L. E. W. Carty, Mrs. M. M. Mathison, and Mrs. A. M. White, and the donation of £10,000 received from the "Sun News-Pictorial" in 1944.

The culmination of Dr. Kellaway's association with the Institute was the transfer to the present buildings in Parkville. The Committee of Management of the Royal Melbourne Hospital accepted the responsibility for the new building, toward the cost of which generous donations were made by Mr. G. R. Nicholas and the family of the late Mr. A. M. Nicholas, as well as by Mr. Russell Grimwade and others. The occupation of the new Institute was a piecemeal process, carried out during the early years of the war. In 1943 Dr. Kellaway resigned to take up the post of Scientific Director of the Wellcome Foundation, and Dr. F. M. Burnet was invited to succeed him in March, 1944.

An organisation such as the Walter and Eliza Hall Institute of Research is dependent largely upon the co-operation and generosity of many other bodies and individuals.

In presenting the 26th Annual Report of the Director, the Board desires to express sincere gratitude to all who have contributed to the funds or have assisted the Institute in other ways. A special tribute is paid to the munificent gift of £10,000 made by The Sun News-Pictorial Ltd., which will allow work of great value to the community to be done by the Institute, and the Board trusts that this generosity will be an inspiration to others.

On behalf of the Board,

B. T. ZWAR, Chairman.

# The Director's Twenty-Sixth Annual Report

TO THE BOARD

OF THE

# Walter and Eliza Hall Institute of Research in Pathology and Medicine

JULY, 1945.

The life of an institution, like that of an organism or a species, is a matter of constant adaption to its changing environment. During the year under review an effort has been in progress to think out and define the new relationships which in one way and another have developed between the Institute, the Hospital, and the University. This year, for the first time, the Annual Report appears without a list of the staff of the diagnostic laboratories of the hospital, and without a report of their year's work. This represents our acceptance with considerable regret of the complete administrative separation of the functions of the diagnostic laboratories and the Hall Institute which has been found desirable by the hospital authorities. At the present time there is little more than physical contiguity between the hospital and the Institute. On the one hand, the major lines of investigation by the staff concern problems which are not those of a general hospital, and on the other, the demands of the war have made it impossible for medical graduates of sufficient calibre to be spared for even part-time clinical research in the wards. It is to be hoped that once demobilisation of the medical services becomes possible, the Institute and the hospital will be able again to make full use of their association by the development of an active programme of clinical research. The appointment of assistant director has been postponed with the object of finding for this position a man primarily interested in clinical problems who could be largely responsible for this development of clinical research within the hospital. If our association with the hospital has been at least temporarily weakened, a more direct link with the University has been forged by my appointment to the newly established Chair of Experimental Medicine.

This appointment is the result of an agreement between the Walter and Eliza Hall Trust, the University of Melbourne, and The Royal Melbourne Hospital, by which the Director becomes ex-officio Professor of Experimental Medicine, and is charged with the organisation of a University department within the Institute. The work of this department will naturally be determined primarily by the interests of the holder of the Chair. The work of the virus department under my direction during the past few years has tended strongly toward an interest in the more general problems of the epidemiology of infectious disease, and it is hoped to develop the University department as one of epidemiology. Its work will complement that of the laboratory workers in the virus department, and it should eventually fill an important niche in the field of medical research and teaching in Melbourne. The University is providing support for the department from the funds of the Haley Trust, and the first active step in its establishment has been the appointment of Miss N. McArthur as Haley Research Officer in Medical Statistics.

We were gratified during the year to receive an offer from the Wellcome Trustees to establish an epidemiological library for use in connection with the Chair of Experimental Medicine. The trustees have undertaken to grant at least £1750 for the purchase of books, and have, in addition, offered to provide help in furnishing and equipping a suitable room in dignified and comfortable fashion. The library which will be concerned primarily with epidemiological material dealing with Australia and New Zealand, will be known as the Wellcome Library of Epidemiological Research. The Board of the Institute has accepted this offer with gratitude and enthusiasm, and the present intention is to locate the library on the 5th floor of the Institute. Plans are now being prepared.

The Board of the Institute has lost the services of its Treasurer through the resignation of Dr. C. H. Mollison. He had represented the medical staff of the hospital on the Board since its inception, and throughout the period his services as treasurer have been of the greatest value to the Institute. We are happy to welcome in his place, as representative of the medical staff, Sir Alan Newton, and we are particularly glad that Mr. H. D. Giddy has found it possible to become treasurer of the Institute. He has been most generous of his time and experience in reorganising the book-keeping of the Institute along lines more suited to its present activities.

If the Institute is to develop as a distinct entity, with a defined relationship to hospital and university, it is desirable that it should have a proper legal existence. After preliminary

discussion with the parties concerned, the Board has decided that the Institute should be suitably incorporated, and the legal process for such incorporation is now under way.

In order to lighten the administrative load carried by the director in the past, Mr. A. Hughes, a former member of our staff, has been appointed Engineer and Business Manager of the Institute. He is responsible for the general control of the building and equipment, and for routine financial matters, salaries, buying and so forth. An adequately equipped workshop is being built up under his supervision.

We have to record with deep appreciation two further large donations toward the work of the Institute. The proprietors of the "Sun News-Pictorial" have presented the sum of £10,000 toward the general funds of the Institute. This gift will be of the greatest value in bringing the equipment of the laboratories up to date, and in making it possible to face with confidence the responsibilities which will devolve upon the Institute in the post-war period.

A gift of £5000 has been received from the trustees of the late Mrs. A. M. White, for the investigation of cancer, tuberculosis and allied diseases. The utilisation of this bequest may be delayed until more normal conditions prevail.

The research work of the Institute has followed the guiding principle that in war-time work should be directed as far as possible to problems of potential value to the armed forces, but that in addition no opportunity should be missed to carry out investigation of fundamental academic problems arising in the course of such work. In the virus department the main practical activity of the year was the organisation of a pilot plant in the Institute for the production on a large scale of influenza virus vaccine. Under Dr. Beveridge's direction this was used both to work out details of the process under local conditions, and to train a nucleus of staff for the Commonwealth Serum Laboratories. Subsequently control was taken over by Dr. J. O'Connor, of the Commonwealth Serum Laboratories, and with the opening of the main plant at the serum laboratories the whole activity was transferred there. The Institute plant produced approximately 30,000 doses of concentrated A and B vaccine for transmission to the United Kingdom.

No influenza was present in southern Australia during the winter of 1944, and investigations on influenza virus were along relatively academic lines. What is hoped will be a valuable method for the isolation of the virus has been developed, the activity of iodine vapour in inactivating droplets of virus at ex-

tremely low concentrations has been observed and studied, and an extensive series of experiments on the Hirst red cell agglutination reaction has been carried out.

The important service problem of nonspecific urethritis was further studied by Dr. Beveridge on the hypothesis that a pleuropneumonia-like organism was responsible for many of the cases. Results have proved very difficult to interpret.

At the request of the Army authorities, Dr. Beveridge made a visit to Central and North Australia to investigate a venereal disease of aborigines, granuloma venereum. The responsible organism was isolated by innoculation of chick embryos, and recommendations as to treatment were made. Dr. Beveridge is now working on the susceptibility of the organism to various chemotherapeutic agents.

By arrangement with the Army Director of Pathology, Col. E. V. Keogh, work on the rickettsial diseases encountered in North Australia by Service personnel has been carried out in the Institute. Majors A. V. Jackson, F. Fenner and J. Funder have been seconded to us for this work for varying periods, and Capt. S. E. Williams has been responsible for much of the field work in North Queensland. It is of very great interest that a type of rickettsia hitherto unknown in Australia has been isolated and is being studied. It appears to be related to the strains responsible for "tick typhus" in various parts of the world.

In the Biochemical Department, Mr. Holden has continued work on haemoglobin derivatives, and has given freely of his experience in providing technical assistance for work in the virus department and for outside bodies. Dr. Gottschalk has made important additions to the theory of the fermentation of sugars by yeast.

## THE VIRUS DEPARTMENT.

*Influenza.*

The winter of 1944 was remarkable for the complete absence of epidemic influenza in Australia. In the groups available to us there was an unusually low incidence of respiratory infection in general, and none of those tested showed any evidence that influenza A or B was concerned. Records were kept of respiratory infections in army personnel who had been immunised with A or B attenuated living vaccine in February. As was to be expected, no difference in the incidence of respiratory infection was found between the two groups.

Laboratory work on influenza virus was, therefore, only indirectly related to the problems of the human disease.

*An Improved Method for Isolation of Virus.*

Owing to difficulties that have been reported by other workers in isolating influenza virus by chick embryo inoculation, we have critically examined the various procedures of amniotic inoculation in order to provide the most satisfactory practical method. Two important points that emerged from the study were, first, that it is possible to inoculate unfiltered throat washings by treating them with penicillin and inoculating concomitantly sodium sulphadiazine into the amniotic cavity, and second, that embryos do not reach full susceptibility to infection by that method until they have been incubated for thirteen days. It was shown that virus could be isolated in full titre from mixtures of influenza virus in the unadapted O phase with unfiltered throat washing from normal persons, and that the same technique could be applied to reisolating virus from infected ferrets. No opportunity to apply it to a natural human epidemic arose.

*Isolation of Virus by Allantoic Inoculation.*

In view of our repeated finding that influenza virus in the form present in the human throat does not multiply in the allantoic cavity, the finding of Thigpen and Rickard, in America, that virus could be isolated by allantoic inoculation appeared to require examination. On making appropriate experiments, it was found possible to confirm the American workers that O virus can be caused to multiply in the allantoic cavity and eventually give rise to freely growing D phase virus. It requires, however, 100-1000 times as much virus to initiate infection in the allantoic cavity as in the amniotic cavity. The amniotic method is, therefore, still the method of choice for primary isolation. In the same series of experiments it was found that as in the allantoic cavity so in the mouse lung our former contention that O virus failed to multiply was too rigid. A certain degree of multiplication can occur when a large dose of O virus is inoculated into mice intranasally, but the method is very much inferior to amniotic inoculation.

*Further Work on O Phase Influenza Virus.*

It has been possible to maintain the O, or human, phase of influenza virus through 24 successive embryo transfers, and to establish a number of points in regard to its behaviour, and to the changes which result in its conversion into the D phase. O virus multiplies preferentially in the chick embryo lung, and if lungs are harvested at an appropriate period high titre O virus material can be obtained. It can be largely purified from D phase virus by absorption with sterile fowl erythrocytes, and if such

absorbed material is stored in a dry ice refrigerator, O virus can be obtained at will by appropriate amniotic inoculation. Between the fully developed O and D phases there is at least one (probably more) intermediate phase or phases. The significance of these is not wholly clear, but all the evidence still points to mutation as the most important factor concerned in the changing character of influenza virus in chick embryo cultures.

*The Melbourne Egg Strain of Influenza Virus.*

In former years the strain of influenza virus "Melbourne" fully adapted to growth on the chorioallantois was used very extensively in this laboratory for immunological work. With the development of the Hirst agglutination test this work lapsed almost completely. Following some preliminary results obtained by Miss Bull before her resignation, I carried out a re-examination of this strain by the more recent methods. The chief results of interest were: (1) The strain has very little power to agglutinate fowl red cells, but agglutinates guinea pig cells to a moderate titre. In this respect, it resembles O phase virus. It differs sharply, however, in failing to agglutinate pigeon cells, which are agglutinated to full titre by O and D phase virus. The strain can from all points of view be regarded as representing a third phase of the same status as O and D. (2) The strain is highly fatal by intravenous inoculation in the embryo infecting at a dose about 1/5 of that necessary to produce a single lesion on the chorioallantois, or to kill by that route. (3) Curiously irregular results follow allantoic inoculation.

*Immunity to Influenza.*

It was found during the previous year that, after one injection of influenza vaccine subcutaneously, people seldom gave any serological response to a second injection. In seeking an explanation of this phenomenon, which is not in keeping with most immunological experience, experiments were conducted in rabbits and mice. It was found that the antigenicity of the virus was destroyed by mixing it with homologous antiserum. Thus the failure to obtain a response to a second inoculation in man is probably due to the circulating antibody resulting from the first inoculation combining with the vaccine before it can reach the antibody forming cells. These findings may also help towards an understanding of the action of adjuvants in enhancing antibody response to influenza vaccines, a recent important development in U.S.A.

*Studies on Haemagglutination by Influenza and Related Viruses.*

Working from the belief that the ability of influenza and some related viruses to unite specifically with the red cells of

various species of animal represents the most important available clue to the fundamental problem of the relationship between virus and cell it infects, several members of the virus department staff have investigated one or other aspects of the reaction.

Mr. Rawlinson has made a detailed study of the substances present in normal allantoic fluid which inhibit the action of the virus on red cells. Methods for accurately estimating the inhibitory activity have been worked out, and a large number of possible substances have been excluded from responsibility for the effect. It has not been possible to isolate the agent concerned, but evidence has been obtained that it is nondiffusible and relatively heat stable. Since the allantoic cavity is surrounded by cells highly susceptible to influenza virus, the possibility that the inhibitory substance found in the fluid is equivalent to shed "receptors" must be seriously considered.

Mr. McCrea is commencing work on the related problem of the inhibitory substances in normal serum. This has some important practical applications since refined serological work with the haemagglutinin test is seriously limited by the existence of variable amounts of normal inhibitor in addition to specific antibody.

During the past three years a number of minor investigations on haemagglutination phenomena have been made incidentally to other work. The results of these have now been collected and submitted for publication as a series of short notes. The most interesting series of experiments provides some information on the nature of the receptors present in the surface of the red cell. It is shown that viruses of the influenza-Newcastle disease group can be arranged in an order such that any given virus will after reacting with red cells leave them insusceptible to that virus and to any virus preceding it in the series while leaving them still partially susceptible to agglutination by viruses following it in the series. The order is—Newcastle disease; Melbourne, GAT; W.S.; BEL; BON, LEE; Swine. The differentiation places the four major antigenic types—Newcastle disease, Influenza A, Influenza B, and Swine influenza viruses in that order, and suggests the existence of a graded series of receptors of decreasing accessibility and increasing firmness of attachment to the cell.

*Iodine and Other Halogens on Influenza Virus.*

Viruses are in general relatively insusceptible to most of the antiseptics and chemotherapeutic agents active against pathogenic bacteria. A noteworthy exception amongst the common antiseptics is iodine, which is highly active in solution against influenza and other viruses. Miss Stone and I have, therefore,

made a fairly extensive study of the action of iodine on influenza virus, with some comparative work with the other halogens, chlorine and bromine.

Three modes of approach have been used—(1) the action of the halogens *in vitro* using the Hirst haemagglutination test as indicator, (2) similar tests with the infectivity for the chick embryo as the indicator of active virus, and (3) the action of known concentrations of iodine vapour on the infectivity of mists of influenza virus for mice.

The destruction of haemagglutinating activity takes place at dilutions of iodine well beyond those causing agglutination or haemolysis of red cells. The other halogens are active at approximately equivalent molecular concentrations. As far as our experience goes the halogens are the only agents which in high dilution destroy the haemagglutinating power and the infectivity of influenza virus *pari passu*. Tests in mice and by inoculation into the allantoic cavity of chick embryos showed that iodine will inactivate typical influenza virus strains at a concentration of $10^{-4.2}$N bromine at $10^{-3.9}$N and chlorine at $10^{-4.0}$N.

The major part of this set of experiments was directed toward the influence of iodine vapour on influenza virus in the form of mist. It is well known that mice can be infected with a suitably active strain of virus by simple exposure to an atmosphere containing suspended virus droplets or dry droplet nuclei. Much work has been done overseas on the sterilisation of such infective mists by various aerosols and vapours. The agents which have been particularly studied are glycol vapours and hypochlorite aerosols. Iodine vapour appears to be considerably more active molecule for molecule than any other agent yet used.

Early non-quantitative experiments showed that prolonged exposure to iodine vapour before or after infection had no significant effect. The mice were protected only by the effect of the iodine vapour on the air-borne influenza virus. A striking indication of the potency of iodine was given by an experiment in which mice were protected merely by painting the outside of their snouts with tincture of iodine a few minutes before they were exposed to mist infection.

Having established that iodine vapour was effective in what were obviously very low concentrations an apparatus for quantitative work was constructed for us by Mr. Hughes. This was a glass reaction chamber of 29 litre capacity, with inlets for the infective mist and for mice. Arrangements were fitted for mixing the air for introducing drying agent and known amounts of iodine, and a trap to allow pressure equalisation without escape of virus into the air. The infective mist was produced by passing

compressed air through an atomiser dipping into a suitable preparation of virus. In most experiments virus concentrated by Francis and Salk's method was diluted in filtered human saliva.

The method of introducing the iodine was due to Mr. Holden. Known small amounts were dissolved in specially purified methanol free from any iodine binding impurities. The amount required in a volume of 0.2-1.0 c.c. of methanol was introduced into the chamber in a small glass stoppered vessel, and the contents mechanically spilt on to a watch glass at the appropriate time and evaporated by the fan.

These experiments showed that the immediate protective concentration against wet virus mist was about 0.5 microgram of iodine per litre, i.e., a concentration of 1 gram of iodine in 2000 million cubic centimetres of air. When the infective mist was dried, resistance to sterilisation was considerably greater, but mice were protected by concentrations of 3 micrograms per litre, provided this was allowed to act on the dried virus mist for 30 minutes. It seems possible that the use of iodine vapour as an air sterilising agent might be of some practical value under emergency conditions in crowded temporary habitations.

*Ectromelia of Mice and Vaccinia Virus.*

A casual observation has led to the unexpected discovery that a specific virus disease of mice, known since 1930 as infectious ectromelia, is due to a virus having a close biological relationship to vaccinia virus. This finding has a curious similarity to Jenner's discovery of the relationship between smallpox and vaccinia (cow-pox) which was the classical first step of virus research. Superficially the viruses of vaccinia and ectromelia produce very different types of lesions, and have a different range of susceptible hosts. There was no obvious reason why any relationship should be expected, and no comparative study has been previously made. Our investigations were initiated by finding that preparations of ectromelia virus agglutinate red cells in the same fashion as vaccinia virus does.

About two years ago, Dr. Nagler found in this Institute that vaccinia virus agglutinates fowl erythrocytes, but only cells from about 50 per cent. of individual birds. This holds, too, for ectromelia virus, cells susceptible to one virus being also susceptible to the other. A significant difference between the two viruses, however, is the susceptibility of mouse cells to agglutination by ectromelia, but not by vaccinia virus.

The further study of this phenomenon has involved work on the nature of the haemagglutinin with Miss Stone, on the serological relations between vaccinia and ectromelia haemag-

glutinins with Mr. W. C. Boake, and on active cross immunity between the two viruses.

The main findings are first that the haemagglutinins of vaccinia and ectromelia are soluble agents distinct from the virus particles themselves in this respect differing sharply from the influenza viruses. It is now firmly established that influenza virus particles are themselves the haemagglutinating agent. Despite this fundamental difference there are some close resemblances between influenza and vaccinia haemagglutinins, e.g., in their heat stability, and in the power of dilute halogen solutions to destroy their activity. A striking difference is our inability to elute the vaccinia or ectromelia haemagglutin from red cells to which it has been attached. The haemagglutin can be removed by the action of immune serum, but no loss of agglutinability of the red cells is detectable by appropriate tests.

Immune sera from animals or human beings that have been infected by either virus show power to inhibit both haemagglutinins. In each case the homologous agent is inhibited to higher titre than the heterologous, but the difference in titres is not large.

Mice can be solidly immunised against ectromelia by a small dose of living vaccinia virus which has practically no pathogenic action on the species. Incomplete experiments have shown no immunising activity with inactive vaccinia virus. Contrary to what is generally stated ectromelia virus has a certain limited virulence for the rabbit. Death may follow a large intravenous injection and large amounts of virus be recoverable from the liver, while relatively concentrated virus injected intradermally gives a lesion resembling a weak vaccinia lesion in the same situation. Rabbits immunised by (non-lethal) intravenous injection of ectromelia fail to react to vaccinia virus given intradermally, and provide serum which inhibits haemagglutination by both viruses. No effective active immunity is produced by subcutaneous immunisation.

Ectromelia of mice can in many respects be regarded as analogous to smallpox in man. It is relatively infectious from mouse to mouse, has a high mortality, and is protected against by immunisation with vaccinia virus. It differs considerably in the distribution of lesions, and in the fact that the results of infection in mice can be divided rather sharply into acutely fatal infections, subacute or chronic infections, and completely subclinical immunising infections. It may be hoped that a close study of the effect of vaccinia virus in protecting mice against ectromelia will provide information relevant to the prevention of smallpox and yellow fever in man.

*Lipoids and Virus Haemagglutination.*

In the course of an attempt to characterise the vaccinia haemagglutination by chemical means alcoholic extracts from virus infected tissues were tested for haemagglutination using two types of fowl cells known to be susceptible and insusceptible to agglutination by vaccinia virus. The extracts showed very high agglutinin titres against susceptible cells and no action on the insusceptible type. The first explanation of this phenomenon, that the virus haemagglutinin was soluble in alcohol, was soon disproved by finding that extracts of normal tissue, as well as preparations of purified cephalin, showed the same differential agglutination of fowl red cells.

The significance of this finding by Miss Stone and Mr. Holden has still to be determined, but it may well mean that lipoids derived either from the infected tissues, or forming an intrinsic part of the virus particle, play an important part in haemagglutination and by inference in the invasion of susceptible tissue cells.

*Nonspecific Urethritis in Soldiers.*

Investigations of this common condition was continued during the year by Dr. Beveridge and Miss Lind. In co-operation with Mr. Campbell, of the Animal Health Research Laboratory of the Council for Scientific and Industrial Research, complement fixation tests were carried out on sera from men with this condition, using an antigen made from strains of pleuropneumonia-like ("P.P.L.") organisms isolated from the diseases. At first the results suggested that the cause of this newly recognised venereal disease had been discovered. Positive results were obtained in 92 per cent. of 62 cases in New South Wales, compared with only 7 per cent. in 100 normal persons in Melbourne. However, subsequently only 37 per cent. positive results were obtained from 46 cases in Victoria. Cultures were made from 70 cases, and from 14 (20 per cent.) of them P.P.L. organisms were isolated, usually in large numbers. Cultures from 25 normal men yielded no positives.

Dr. Ella MacKnight co-operated in the investigation by examining a number of women from whom the men had contracted the disease. Cultures positive for P.P.L. organisms were obtained from three out of eleven of these. Dr. MacKnight also collected swabs from 101 normal women. Positive cultures were obtained from 17 of these. These strains showed no obvious cultural differences from those obtained from men with urethritis.

These results necessitate the abandonment of the hypothesis that P.P.L. organisms are the specific casual agent of most

cases of so-called nonspecific urethritis. Nevertheless, they may have some pathogenic role, at least in those cases from which they can be cultured in large numbers. In the female genital tract they are apparently harmless saprophytes, but this may be analogous to the frequent presence of potentially pathogenic pneumococci in the nose of normal persons.

Various substances were tested out for their germicidal action on P.P.L. organisms in the presence of serum. The only ones with any effect under the conditions of the test were Zephiran and C.T.A.B. These have been tested on some 20 cases of the disease with apparently excellent results. However, there were no controls.

Cultures have also been made for P.P.L. organisms from 57 infected antra, 70 excised tonsils, 4 cases of atypical pneumonia, and a few other specimens from infections of unknown cause. No positive results have been obtained.

Sera from two cases of Northern Territory arthritis did not give positive complement fixation test to P.P.L. antigen.

*Granuloma Venereum.*

By arrangement with, and at the request of, the Australian Army, Dr. Beveridge went to the Northern Territory to investigate this tropical venereal disease, which occurs in the natives there. This disease is caused by an organism which is morphologically like bacteria but cannot be cultivated by bacteriological methods. A recent report from U.S.A. indicated that the organism could be grown in the yolk sac of the chick embryo. Two strains were isolated in the Northern Territory by inoculation of eggs, together with penicillin to suppress the growth of contaminants. These strains have now been carried through numerous passages, and have shown the same general characteristics as have been described for the American strains.

Tests have been conducted in eggs to determine the most effective antimony compound for chemotherapy against this disease. Trivalent compounds have been found to inhibit the multiplication of the organism in 1/10th the concentration that is required with pentavalent compounds. One millionth of a gramme of trivalent antimony per egg prevents growth, i.e., a dilution of about 1 in 20,000,000 in the yolk. It inhibits in embryonic yolk in the absence of living cells at a dilution of about 1 in 1,000,000, but as much as 1 in 10,000 does not kill the organism in two days.

The action of antimony has been found to be prevented by certain reducing compounds, e.g., thioglycolic acid or ascorbic acid. This may explain why poor clinical results were obtained

in the Northern Territory, when adequate local antibacterial treatment was not carried out at the same time as the antimony therapy, for such reducing compounds are probably produced by bacterial infections. When antibacterial measures were instituted the cases responded well to antimony treatment. This bears some analogy to interference with the action of sulphonamides by para-aminobenzoic acid produced by well established local infections.

Sulphadiazine showed no effect against the Donovan bodies in the egg.

The virus of lymphogranuloma was not inhibited in eggs by antimony compounds.

*Penicillin and Anaerobic Infections.*

Dr. Nagler has continued his work on the susceptibility of anaerobic organisms to penicillin. He has shown in his experiments that when treatment is begun six hours after the initiation of infection with *Clostridium welchii* neither penicillin nor antitoxin alone will prevent death of most of the animals. By the use of a combination of the two it has been possible to protect nearly 100 per cent. of the experimental animals for the three day period over which treatment was continued, and to allow indefinite survival for a variable proportion (up to 73 per cent.). The degree of success attained depended largely on the amount of penicillin given. The dosage required over a three day period corresponds approximately to a total dosage of 3 to 4 million Oxford units of penicillin and 150,000 units of antitoxin for an adult human patient.

Dr. Nagler carried out investigations on the action of penicillinase on penicillin, with the object of devising a more suitable and reliable method for the estimation of the latter. However, no parallelism could be established in the action of penicillinase on penicillin. This probably indicates that the enzyme activity of penicillinase is of a complex character.

Dr. Nagler is now studying experimental staphylococcal osteomyelitis in rabbits and its treatment with penicillin.

*Investigations by A.A.M.C. Officers of Fevers in Tropical Australia.*

During the year we have co-operated with the Army in the study of a number of examples of fevers in tropical areas of Australia. By arrangement with the Director of Pathology, Col. E. V. Keogh, there has usually been one or two officers working in the institute in close association with others concerned with field studies. In the course of this work several interesting findings

have been made. Major P. DeBurgh, in studying a north Queensland fever, inoculated blood intraperitoneally into mice. By repeated passage from these mice he obtained a virus which was sent to Melbourne for further study. The virus showed the large elementary bodies characteristic of the psittacosis lymphogranuloma group, and in every respect behaved like a typical psittacosis virus. At first it was thought possible that the virus had actually been isolated from the original human patient's blood, but further study by Capt. S. E. Williams indicated that the organism had been derived from an infection of the laboratory mice that were used. The virus, like psittacosis virus, produces severe intraperitoneal infections, and is resistant to sulphadiazine therapy. In both these respects it differs sharply from the previously described mouse viruses of this group, and provides a warning against the danger of false diagnoses of psittacosis when mouse inoculation is used.

In the latter half of 1944 a group of cases of typhus-like fever occurred amongst troops on exercises in North Queensland. These cases were clinically fairly mild, showed an eschar not unlike that of scrub typhus, but produced no significant rise in OXK agglutinins, and a moderate development of OX 19 agglutinin. A number of the cases gave a circumstantial history of tick bite, and in one instance, a tick was picked off from a spot at which an eschar subsequently developed. The exercises were carried out in rain-forest country, and tick bite was frequent.

From two cases of this type blood inoculations into mice and guinea pigs allowed the isolation of two strains of rickettsia "Fishlock" and "Phillips," by Capt. Williams and Major Jackson. These strains are now under study in guinea pig and chick embryo passage.

About the same time a small group of mild typhus-like cases occurred in urban areas in Queensland which had all the characteristics of murine typhus. Two successful isolations of rickettsiae from the blood of these patients were made, and these strains "Paton" and "Jones" are being studied in comparison with those from the presumed tick typhus cases "Fishlock" and "Phillips."

Sera from the various patients concerned in these outbreaks were sent to Washington for study by Col. Plotz, of the U.S. Typhus Commission. His results showed that the cases "Paton" and "Jones" were typical murine typhus, but the serum from several cases of presumed tick typhus gave no serological reactions with any of the rickettsiae that were available. This

would indicate that the infection is due either to an antigenically new type of rickettsia or to an organism corresponding to one of the other tick bite fevers which have not yet been fully investigated. In the Institute, Major Jackson and Major Funder have studied differences between these two groups of rickettsiae in regard to their pathogenicity for guinea pigs and chick embryos. In the guinea pig, both types of rickettsiae produced rather similar mild infections. After intraperitoneal inoculation a low grade fever appears, with an average incubation period of 4-5 days. With both types a scrotal reaction develops, involving mainly the tunica vaginalis with fibrino-purulent exudate containing easily visible rickettsiae. Cross immunity tests in guinea pigs have so far given indefinite results, but there is certainly no complete cross immunity between the groups.

The murine strains have been shown to persist in the brain of rats following intraperitoneal inoculation for at least 63 days in the case of "Jones" strain, and for 32 days in the case of the "Paton" strain. Attempts to recover the "Fishlock" and "Phillips" strains from the brains of rats similarly inoculated have consistently failed. Tests were made at intervals from 13 to 63 days after intraperitoneal inoculation. This failure to become established in the brain of the rat is characteristic of rickettsiae from tick typhus in other parts of the world.

Both types grow fairly readily in the yolk sac of the chick embryo. The murine strains grow more profusely at 37° than at 32°C., and cause death of the embryo most frequently on the fourth and fifth day, but often later. Strains "Fishlock" and "Phillips" grow better at 32°C. than at 37°C., and the embryo rarely survives beyond the fourth day, and death may occur as early as the second. Under the optimal temperature conditions rickettsial growth has been much more abundant with the murine strains than with those from the presumptive tick typhus. So far growth of the latter strains has not been sufficient to allow the preparation of rickettsial emulsions for serological work. Cultures on Zinsser's agar tissue culture medium have shown more promise for this purpose and are being actively studied. It is hoped to undertake investigations on the mode of transfer of the disease and preliminary work in establishing a colony of the tick *Ixodes holocyclus* is under way.

Dr. Beveridge's work on the organism of granuloma venereum is referred to in another paragraph. There were, in addition, two unsuccessful attempts to isolate microorganism from cases of fever of uncertain origin in Queensland.

*Publications.*

W. I. B. Beveridge:

"The Effect of the Temperature of Preliminary Incubation on Susceptibility of the Chick Embryo to Influenza Viruses." The Australian Journal of Experimental Biology and Medical Science, 1944, 22, 169.

"Factors Determining the Incidence of Influenza and the Interval Between Repeated Attacks in the Same Person," The Australian Journal of Science, 1945, 7, 137.

"Lack of Increase in Antibody after Second Injection of Influenza Virus in Man." The Australian Journal of Experimental Biology and Medical Science, 1944, 22, 301.

W. I. B. Beveridge, Joyce D. Stone and Patricia E. Lind.

"Suppression of Antigenicity of Influenza Virus by Admixture with Homologous Antiserum." The Australian Journal of Experimental Biology and Medical Science, 1944, 22, 307.

F. M. Burnet:

"Influenza and Other Respiratory Infections." Medical Journal of Australia, 1944, 2, 1.

"Medical Education and Research: Impressions of an American Visit." Medical Journal of Australia, 1944, 1, 557.

"Some Borderlands of Microbiology, Genetics and Biochemistry." Australian Journal of Experimental Biology and Medical Science, 1944, 7, 1.

"An Unsuspected Relationship Between the Viruses of Vaccinia and Infectious Ectromelia of Mice." Nature (In the press).

"Poliomyelitis in the Light of Recent Experimental Work." Health Bulletin (Victoria), January-June, 1945.

F. M. Burnet, W. I. B. Beveridge, J.McEwin and W. C. Boake.

"Studies on the Hirst Haemagglutination Reaction with Influenza and Newcastle Disease Virus" (In the press).

1. "Partial Dissociation of Haemagglutin and Infective Activity of Newcastle Disease Virus."
2. "Experiments on the Elution of Newcastle Disease Virus and Influenza Virus from Fowl Cells."
3. "The Process of Virus Neutralisation as Observed with the Hirst Haemagglutination Method."
4. "The Action of Human Tears on Influenza Virus."
5. "Agglutination of Pigeon Erythrocytes by Influenza Virus A in the O Phase."

F. M. Burnet and W. C. Boake:

"The Relationship Between the Virus of Infectious Ectromelia of Mice and Vaccinia Virus." Journal of Immunology (In the press).

F. M. Burnet and D. R. Bull:
"Re-examination of the Influenza Virus Strain 'Melbourne Egg.'" Australian Journal of Experimental Biology and Medical Science, 1944, 22, 173.

F. M. Burnet and J. D. Stone:
"The Significance of Primary Isolation of Influenza Virus by Inoculation of Mice or of the Allantoic Cavity of Chick Embryos." Australian Journal of Experimental Biology and Medical Science, 1945, 23, 147.
"A Method for the Isolation of Influenza Virus from Throat Washings Without Filtration." Australian Journal of Experimental Biology and Medical Science, 1945, 23, 161.
"Further Studies on the O-D Change in Influenza Virus A." Australian Journal Experimental Biology and Medical Science, 1945, 23, 151.

F. P. O. Nagler:
"A Cultural Reaction for the Early Diagnosis of *Clostridium oedematiens* Infections." Australian Journal of Experimental Biology and Medical Science, 1945, 23, 59.
"Treatment of Experimental Gas Gangrene Due to *Clostridium welchii* with Penicillin and Antitoxin." The British Journal of Experimental Pathology, 1945 (In the press).

P. DeBurgh, A. V. Jackson and F. E. Williams:
"Spontaneous Infection of Laboratory Mice with a psittacosis-like Organism" (In the press).

## BIOCHEMICAL DEPARTMENT.

During the year 1944-45 Mr. Holden has continued his experiments on "cruoralbin," a green pigment formed by the repeated reduction and reoxidation of cyan-methaemaglobin or oxyhaemaglobin in the presence of hydrogen cyanide. They have been directed towards two main objects—the isolation and investigation of the prosthetic group of cruoralbin, and the establshment of the relationships beween cruoralbin and the other greenish pigments derived from haemoglobin, e.g., sulphaemaglobin and choleglobin.

The ability of cruoralbin and of sulphaemoglobin to combine with carbon monoxide was demonstrated by analysis of their compounds using a sensitive method for carbon monoxide determination devised in this Institute. Horse sulphaemoglobin was crystallised, but horse cruoralbin has as yet resisted all attempts to crystallise it. The prosthetic group ("cruoratin") of cruoralbin has been detached from the protein. It is very un-

stable, and has not been prepared pure in the solid state. Its ultra-violet spectrum shows the Soret band and hence its porphin ring is apparently intact. Its iron atom is more readily detached than that of haematin.

Unlike choleglobin, cruoralbin and cruoratin do not yield bile pigments when treated with acetic acid and ether. Little of the iron of cruoralbin, in contrast to that of choleglobin, and of pseudo-haemoglobin, is detached by heating to 37°C. with decinormal hydrochloric acid.

In general it would appear that as yet the structure of none of the "green pigments" is well ascertained, and their part in the physiology or pathology or haemoglobin is far from clear. During the year hospitality has been afforded to No. 1 Flying Personnel Research Unit, R.A.A.F., for the determination of carbon monoxide in specimens of air and of blood. The initial stages of some work involving determinations of hydrogen-ions was also done here.

*Fermentation of Fructose and Other Sugars.*

Dr. Gottschalk continued his investigation of the physical and chemical factors determining the biological utilisation of d-fructose. As previously shown beta d-fructopyranose, the only crystalline form of d-fructose, is unfermentable by yeast. From this it was concluded that d-fructofuranose, into which part of the fructopyranose is converted on solution in water (mutarotation) is the fermentable fructose modification. Experiments with raffinose and with sucrose as fermentation substrates have justified this deduction: (a) 0.1 M Raffinose, split by baker's yeast into fructofuranose and the unfermentable melibiose, is freely fermented at 0°C. and pH 4.5 under which conditions mutarotation proceeds at its lowest rate. (b) When the fermentation by baker's yeast at 0°C. of 0.002 M sucrose, which is immediately hydrolysed into equal parts of alpha d-glucose and beta d-fructofuranose, is interrupted after 20 minutes, about the same amounts of aldohexose and ketohexose are recovered.

The fermentation rates by brewer's and baker's yeast of d-fructofuranose and of d-glucose conform to the theorem of Michaelis and Menten. The ratio of the maximum velocities Vf/Vg equals 1.05. It is well known, however, that Sauternes yeast (Sacch. ellipsoideus) ferments fructose much faster than glucose even at high concentrations of the substrates (up to 0.5 M conc.). Recently we observed a similar behaviour with champagne yeast (Sacch. ellips.). These differences, however, between brewery and champagne yeasts in their behaviour towards the keto- and the aldehyde sugar disappear when dried preparations

of the yeast are used. Both with dried brewery and champagne yeasts, it was found that at 0.027 M initial phosphate concentration the maximum fermentation rates of fructofuranose and of glucose (both in excess) approach very closely; at 0.081 M phosphate concentration the ratio of the maximum rates is about 1.3.

With optimal orthophosphate and hexose concentrations the different maximum rates of fructofuranose and of glucose fermentation by dried yeast indicate different rates of the hexokinase reaction with fructofuranose and with glucose. It would appear that in the presence of excess substrate the turnover of the phosphorylation of fructofuranose by hexokinase is about 30 per cent. greater than that of glucose. The greater reactivity of fructofuranose with hexokinase reveals itself most noticeably at low concentrations of the substrate. Thus, using dried brewery yeast and 0.081 M phosphate concentration with fructofuranose half the maximum velocity was attained at $3 \times 10^{-3}$ M concentration as compared with glucose at $7.2 \times 10^{-3}$ M concentration. This is in close agreement with the results of measurements using living yeast cells and showing that the ratio of the dissociation constant of the hexokinase-glucose complex to that of the hexokinase-fructofuranose complex is approximately 2.

These results allow a more uniform interpretation of the initial step in alcoholic fermentation of d-glucose and d-fructose by different types of yeast than was possible in last year's report. The almost identical behaviour of dried brewery and champagne yeasts towards glucose and fructofuranose strongly suggests that their different behaviour in the living state is due to differences in the permeability of their respective cell membranes for the two sugars rather than to differences in the enzymes concerned. It would thus appear that the same enzyme, hexokinase, catalyzes the phosphate transfer from ATP to the aldo- and ketohexose, with the difference, however, that the enzyme acts upon all d-glucose modifications, whereas of the various d-fructose forms only fructofuranose is a suitable substrate. This very reactive and labile substance has about twice the affinity of glucose for hexokinase.

The group specificity of hexokinase for d-fructofuranose, d-glucose and d-mannose relates to their common molecular structure at carbon atoms 3, 4, 5 and 6; the span of the oxygen bridge (aldohexopyranoses, ketohexofuranoses) and the configuration at $C_1$ (alpha, beta isomers) and $C_2$ (glucose, mannose) are only factors determining the affinity of the respective substrates for hexokinase and their turnover by this enzyme.

In the course of the year Dr. Gottschalk carried out halogen determinations for the Virus Department, and gave some advice to the fermentation industry.

A. Gottschalk:

"The Kinetics of Fermentation of d-Fructofuranose by Yeast." The Australian Journal of Experimental Biology and Medical Science, 1944, 22, 291.

## DEPARTMENT OF CLINICAL RESEARCH.

*Tests for the Services.*

Blood grouping tests for the Services have totalled 11,458, a further big fall from the number done in the earlier years of the war.

Miss Williams has been responsible for 1745 complement fixation tests during the year, the majority of them being done for the R.A.A.F. or A.M.F. Wassermann tests comprised about three-quarters of the total, with small numbers of gonococcal and hydatid tests.

*Complement Fixation Tests in Malaria and Schistosomiasis.*

At the request of Brigadier N. H. Fairley, Miss Williams has carried out investigations on the complement fixation test in malaria, using both *Pl. gallinaceum* and *Pl. knowlesi* antigens. The antigens were prepared in America. Patients from Heidelberg Military Hospital known to have had malaria gave 73/207 positive reactions (35 per cent.) with *Pl. gallinaceum* antigen. A number of these sera negative to *Pl. gallinaceum* gave a positive reaction with *Pl. knowlesi.*

More systematic tests with sera received from L.H.Q. Malaria Research Unit were made with *Pl. knowlesi* antigen. The results will be used by the staff of the Unit.

Cases of infection with *Schistosomum japonicum* have been reported from the Pacific operational areas, and nine sera from suspected cases were tested by Miss Williams. The antigen used was sent from India in 1926 by Dr. N. H. Fairley. It is an alcoholic extract made from livers of the snail *Planorbis exustus* heavily infested with *Schistosomum spindalis.* It has been extensively used for the diagnosis of *S. mansoni and S. haematobrium* infections, but this was the first opportunity to use it with *S. japonicum* infections. Of nine sera tested, seven were strongly positive, three fixing more than 35 M.H.D's. of complement. The sera sent were from patients in whom the diagnosis was made largely on the basis of a persistent cosinophilia. It is

of interest that the two serologically negative cases were later found to be infected with hookworm.

*Red Cross Blood Transfusion Service.*

Only the serological work in connection with the Red Cross Blood Transfusion Service is now being carried on in the Institute. For the year ending 1/5/45 the blood samples of 624 newly enlisted donors were tested for their blood groups and subgroups, Rh factor, serum titre, haemoglobin value and Kahn reaction.

For various reasons, 148 former donors were retested both in regard to blood characteristics and for their medical and general suitability as blood donors. Sera from a large proportion of previously tested donors were subjected to the Kahn test, and 5546 haemoglobin estimations were carried out on the blood of women donors who had given more than one blood donation. Those whose haemoglobin was found to be under 11.9 grams haemoglobin per cent. have been referred for treatment.

Two hundred and fifty-nine batches of pooled serum were tested for their isoagglutinin titre. Eight hundred and seventeen additional Kahn tests were done on blood samples sent to the Institute for Wassermann reaction, or in connection with investigations on abortions, stillbirth and sterility.

Four hundred and fifty blood samples were sent by obstetricians and medical practitioners for blood group and Rh determination. Blood samples of 92 married couples with suggestive histories were examined for possible blood incompatibility.

*Investigations on Rh Factor.*

Dr. Jakobowicz has carried out extensive investigations in collaboration with the staff of the Queen Victoria Hospital on the serological relationship betwen mother and infant with reference particularly to Rh incompatibility. She has also done a large amount of similar work for other obstetricians.

A study of complications in the infant which might be related to abnormal blood destruction or other immunological incompatibility, gave very little evidence that either Rh incompatibility or A.B.O. incompatibility produced many such complications. Where mother and child were of the same Rh group 23/311 (7.4 per cent.) showed complications; when the mother was Rh negative, and the child Rh positive, 5/37 complications (13.5 per cent.), and when the positions were reversed 3/36 (8.3 per cent.). The two cases of icterus gravis found in the series of 384 completed pregnancies were (1) in an Rh+ child of an Rh

—mother, and (2) in a Group A child of a Group O mother, both being Rh +.

No significant difference was found in the proportion of foetal or neonatal deaths from Rh+ and Rh— mothers respectively.

Tests for Rh group have now been made on 10,000 persons in Victoria, the percentage of Rh— being 16.4 per cent. in agreement with findings elsewhere.

## LIBRARY.

Our thanks for the gifts of journals and books are due to the following:—B.M.A. (New Zealand Branch); The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; Imperial Chemical Industries, London; Dr. C. H. Kellaway, F.R.S.; The London Hospital; The Medical Research Council; The Middlesex Hospital Medical School; New York Academy of Medicine; New York State Department of Health, Division of Laboratories and Research; Department of Pathology, University of Oxford; Rockefeller Institute, New York; The South African Institute for Medical Research; U.S. Public Health Service; The University of Harvard, Department of Tropical Medicine; University of Leeds; and the University of Pennsylvania, Department of Pathology; University of Texas School of Medicine.

## Donations and Subscriptions for the Year Ended 30th June, 1945.

| | | | |
|---|---|---|---|
| "Sun News-Pictorial" | £10,000 | 0 | 0 |
| Estate of the late A. M. White, per Union Trustees Co. of Australia Ltd. | 5,000 | 0 | 0 |
| The Truby and Florence Williams Charitable Trust, per Trustees, Executors and Agency Co. Ltd. | 1,000 | 0 | 0 |
| Trustees of A. Felton Bequest | 270 | 0 | 0 |
| Estate of late A. Mackie | 150 | 0 | 0 |
| Alfred Edments Trust | 100 | 0 | 0 |
| Diecasters Ltd. | 100 | 0 | 0 |
| Estate of late R. J. Forbes | 50 | 0 | 0 |
| Australian Oxygen and Industrial Gases Ltd. | 25 | 0 | 0 |
| Estate of late E. M. Carty | 21 | 4 | 0 |
| National Bank of Australasia Ltd. | 10 | 10 | 0 |
| Colonial Sugar Refining Co. Ltd. | 10 | 10 | 0 |
| M. M. Brodie | 5 | 5 | 0 |
| Mr. and Mrs. H. D. Giddy | 5 | 5 | 0 |
| A. J. Whalley | 5 | 5 | 0 |
| J. Monahan Lewis | 3 | 3 | 0 |
| Christy Products Pty. Ltd. | 2 | 2 | 0 |
| Brooklands Accessories Ltd. | 2 | 2 | 0 |
| Estate of C. Z. Taylor | 0 | 3 | 6 |
| | £16,760 | 9 | 6 |

### DORA LUSH MEMORIAL FUND.

| | | | | | | |
|---|---|---|---|---|---|---|
| Funds in hand at 30th June, 1944 | | | | £610 | 0 | 0 |
| Received during year ended 30th June, 1945.— | | | | | | |
| A Group of Friends | £40 | 9 | 5 | | | |
| M. Hedderwick | 2 | 2 | 0 | | | |
| Mrs. Ogilvie | 1 | 11 | 6 | | | |
| Mrs. G. Cook | 1 | 1 | 0 | | | |
| G. V. Hirst | 1 | 1 | 0 | | | |
| J. McEvoy | 1 | 1 | 0 | | | |
| E. Le Galliene | 1 | 1 | 0 | | | |
| B. O'Connor | 1 | 1 | 0 | | | |
| | | | | 49 | 7 | 11 |
| Total at 30th June, 1945 | | | | £659 | 7 | 11 |

### AUDITOR'S CERTIFICATE.

I beg to report that I have audited the Accounts of the Walter and Eliza Hall Institute of Research in Pathology and Medicine for the year ended 30th June, 1945. I have obtained all the information and explanations required in the course of Audit, and I am of the opinion that the Balance Sheet and Statement of Receipts and Expenditure are drawn up so as to exhibit a true and correct view of the Institute's affairs, and of the result of the business of the Institute for the year, according to the best of my information, and the explanations given to me, and as shown by the books of the Institute.

(Signed) W. M. JARVIE, F.C.A. (Aust.).
Auditor.

# The Walter and Eliza Hall Institute of Research in Pathology and Medicine

**Balance Sheet at 30th June, 1945.**

## LIABILITIES.

| | | | | | | |
|---|---|---|---|---|---|---|
| General Funds of the Institute | | | | £35,290 | 15 | 2 |
| Dora Lush Memorial Fund | | | | 659 | 7 | 11 |
| National Health and Medical Research Council— | | | | | | |
| Funds at 30th June, 1944 | £495 | 5 | 0 | | | |
| Grants for Year— | | | | | | |
| General | 978 | 5 | 0 | | | |
| Virus | 3,425 | 0 | 0 | | | |
| | £4,898 | 10 | 0 | | | |
| Expended on Salaries and Materials | 4 682 | 14 | 9 | | | |
| Carried forward | | | | 215 | 15 | 3 |
| | | | | £36,165 | 18 | 4 |
| E. Marion Carty Trust Fund— | | | | | | |
| As at 30th June, 1944 | £4,531 | 3 | 6 | | | |
| Add Surplus on Conversion of Securities | 265 | 19 | 6 | | | |
| | | | | 4,797 | 3 | 0 |
| G. C. M. Mathison Trust Fund— | | | | | | |
| As at 30th June, 1944 | | | | 6,500 | 0 | 0 |

## ASSETS.

| | | | | | | |
|---|---|---|---|---|---|---|
| Investments on General Account— | | | | | | |
| Australian Consolidated Inscribed Stock, Face Value £18,580; cost | £18,580 | 0 | 0 | | | |
| Melbourne & Metropolitan Board of Works Inscribed Stock, Face Value, £5,800; Cost | 5,743 | 8 | 6 | | | |
| City of Melbourne Inscribed Stock, Face Value £3,800; Cost | 3,800 | 0 | 0 | | | |
| Melbourne Harbour Trust Inscribed Stock, Face Value, £500; Cost | 500 | 0 | 0 | | | |
| State Savings Bank Credit Foncier Debenture Stock, Face Value £1,500; Cost | 1,496 | 5 | 0 | | | |
| Fixed Deposit, Bank of New South Wales | 63 | 16 | 9 | | | |
| | | | | £30,183 | 10 | 3 |
| Cash Accounts— | | | | | | |
| English, Scottish & Australian Bank Ltd., North Melbourne, Trust Account | £100 | 0 | 0 | | | |
| Agent-General, London | 24 | 14 | 7 | | | |
| Bank of New South Wales, Melbourne | 5,857 | 13 | 6 | | | |
| | | | | 5,982 | 8 | 1 |
| | | | | £36,165 | 18 | 4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | £47,463 1 4 |
| E. Marion Carty Trust Fund Investments— | | | | | | |
| Australian Consolidated Inscribed Stock, Face Value £4,490; Cost | £4,490 | 0 | 0 | | | |
| Melbourne & Metropolitan Board of Works Inscribed Stock, Face Value £250; Cost . . . . . | 250 | 0 | 0 | | | |
| Fixed Deposit, Bank of New South Wales . . . . . | 54 | 15 | 0 | | | |
| Bank of New South Wales, Current Account . . . . . | 2 | 8 | 0 | | | |
| | | | | 4,797 | 3 | 0 |
| G. C. M. Mathison Trust Fund Investments— | | | | | | |
| Australian Consolidated Inscribed Stock, Face Value £6,500; Cost . . . . . | | | | 6,500 | 0 | 0 |
| | | | | £47,463 | 1 | 4 |

NOTE.—No account is taken in the above Statement for the value of apparatus, fittings or equipment of the Institute.

## Statement of Receipts and Expenditure for the Year Ended 30th June, 1945.

### RECEIPTS.

| | | | |
|---|---|---|---|
| To Balances to credit of Bank Accounts— | | | |
| 1st July, 1944 | | £4,128 0 7 | |
| „ Grants— | | | |
| Walter & Eliza Hall Trustees | £3,200 0 0 | | |
| Department of War Organisation of Industry Gas Gangrene Research | 500 0 0 | | |
| University of Melbourne | 750 0 0 | | |
| National Health and Medical Research Council | 978 5 0 | | |
| Do., Virus Account | 3,425 0 0 | | |
| | | 8,853 5 0 | |
| „ Donations and Subscriptions— | | | |
| As per Statement on page 29 | 16,760 9 6 | | |
| Dora Lush Memorial Fund | 49 7 11 | | |
| | | 16,809 17 5 | |
| „ Fees received for services | | 2,956 16 0 | |
| „ Proceeds Sale of Publication | | 103 5 9 | |
| „ Income of Investments | | 762 6 8 | |
| „ Bank of New South Wales, Fixed Deposit Repaid | | 4,002 16 0 | |
| „ Received on Transfer of Securities | | 2 8 0 | |
| | | £37,618 15 5 | |

### EXPENDITURE.

| | | | |
|---|---|---|---|
| By National Health and Medical Research Council, General Account— | | | |
| Salaries and Materials | £1,257 14 9 | | |
| „ National Health and Medical Research Council, Virus Account— | | | |
| Salaries and Materials | 3,425 0 0 | | |
| „ G. C. M. Mathison Fellowship— | | | |
| Salary Paid | £211 5 0 | | |
| Income of Trust Fund | 211 5 0 | | |
| „ E. M. Carty Fellowship— | | | |
| Salary Paid | £412 10 0 | | |
| Income of Trust Fund | 175 13 9 | | |
| | | 236 16 3 | |
| „ Salaries and Wages— | | | £4,919 11 0 |
| Professional | 3,875 13 10 | | |
| Secretarial | 502 5 0 | | |
| Assistants | 2,092 6 10 | | |
| | | 6,470 5 8 | |
| „ Pay Roll Tax | | | 220 13 7 |
| (Total Salaries and Wages paid by Institute for the Year ended 30/6/45, £9,986/5/0.) | | | |
| „ Materials | | | 2,235 4 2 |
| „ Apparatus | | | 173 10 5 |
| „ General Expenses | | | 588 17 3 |
| „ General Maintenance, Power, Light, Etc. | | | 1,570 0 2 |
| „ Library Purchases | | | 283 3 1 |
| „ Fittings and Equipment | | | 121 8 10 |
| „ Petty Cash Expenditure | | | 175 19 9 |
| | | | £16,758 13 11 |
| „ Investments— | | | |
| £5000 3¼% 15/10/1960 Australian Consol. Stock (Estate late A. M. White) | £5,000 0 0 | | |
| £5000 3¼% 15/10/1961, Australian Consol. Stock (ex General Funds) | 5,000 0 0 | | |
| £5000 3¼% Advance Subscription (ex General Funds) | 5,000 0 0 | | |
| | | 15,000 0 0 | |
| „ Balance to Credit of Bank Account | | 5,860 1 6 | |
| | | £37,618 15 5 | |